Capillary Electrophoresis & Troubleshooting

John M. Butler
NIST Applied Genetics Group
National Institute of Standards and Technology
Gaithersburg, Maryland

Presentation Outline

• History and background on CE
• Fundamentals of CE
  – sample prep, injection, separation, detection
• ABI 3500
• Troubleshooting strategies and solutions
• Questions

My Goal:
To help you understand the basic chemistry behind DNA separations and to help make CE instruments less of a “black box”

NIST and NIJ Disclaimer
Funding: Interagency Agreement between the National Institute of Justice and NIST Office of Law Enforcement Standards

Points of view are mine and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

Our publications and presentations are made available at: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Steps Involved

Steps in DNA Analysis

Blood Stain Buccal swab
Sample Collection & Storage

DNA Extraction Quantitation

STR Typing

Interpretation of Results

Database Storage & Searching

Calculation of Match Probability

Stellan Hjertén
Uppsala University (Sweden)

1967
First high voltage CE system (with rotating 3 mm i.d. capillaries)

1981
First “modern” CE experiments (with 75 µm i.d. capillaries)

1988/90
First DNA separations in a capillary (gel-filled/ sieving polymer)

With first fully automated capillary free zone electrophoresis apparatus in 1967

Received his PhD (1967) under Professor Arne Tiselius who had developed moving boundary zone electrophoresis in 1937 (Nobel Prize in 1948)

In 2003 at age 75

Pioneers of Capillary Electrophoresis

Stellan Hjertén
Uppsala University

James Jorgenson
University of North Carolina

Barry Karger
Northeastern University

1967

1981

1988/90

1967

1981

1988/90

1967

1981

1988/90

http://www.rsc.org/delivery/_ArticleLinking/DisplayArticleForFree.cfm?doi=b307798p&JournalCode=AN

Uppsala University (Sweden)

Received his PhD (1967) under Professor Arne Tiselius who had developed moving boundary zone electrophoresis in 1937 (Nobel Prize in 1948)
A Brief History of Capillary Electrophoresis

- 1937 – Tiselius develops moving boundary electrophoresis
- 1967 – Hjertén uses rotating 3 mm i.d. tubes for CE
- 1981 – Jorgenson and Lukacs demonstrate first high performance CE separations with 75 µm i.d. capillary
- 1988 – Karger’s group shows DNA separations of single stranded oligonucleotides with gel-filled capillaries
- 1990 – Karger’s group shows DNA separations with sieving polymers on DNA restriction fragments
- 1991 – Grossman expands work with sieving polymers
- 1992 – Bruce McCord starts working on PCR product separations with STR allelic ladders

My Experience with CE, STRs, etc.

- May 1993 – began working in Bruce McCord’s lab at Quantico
- Sept 1993 – developed mtDNA amplicon quantitation method (used in FBI casework from 1996 to present)
- Nov 1993 – first demonstration of STR typing by CE (using dual internal standards and TH01 ladder)
- July 1995 – defended Ph.D. dissertation entitled “Sizing and Quantitation of Polymerase Chain Reaction Products by Capillary Electrophoresis for Use in DNA Typing”
- July 1995 – ABI 310 Genetic Analyzer was released

First Rapid STR Typing with Capillary Electrophoresis

Single color detection with dual internal size standards
Butler et al. (1994) BioTechniques 17: 1062-1070

My Experience with CE, STRs, etc. (cont.)

- 1996-1997 Developed STRBase while a postdoc at NIST
- Nov 1998 – GeneTrace Systems purchased a 310; typed several hundred samples with Profiler Plus and Cofiler kits and compared results to mass spec STR analysis
- 1999-present – Run thousands of samples with all STR kits available (except PP 1.2) and developed a number of new STR multiplex systems
- Jan 2001 – Published “Forensic DNA Typing: Biology and Technology behind STR Markers” (2nd Edition in Feb 2005)
- April 2001-present – Use of ABI 3100 16-capillary array system

Why Use CE for DNA Analysis?

1. Injection, separation, and detection are automated.
2. Rapid separations are possible
3. Excellent sensitivity and resolution
4. The time at which any band elutes is precisely determined
5. Peak information is automatically stored for easy retrieval

Symbol first used in Oct 1994 at the Promega meeting when I had a poster introducing the use of CE for STR typing
Important Differences Between CE and Gels

- **Room temperature control** is essential for run-to-run precision
  - CE uses sequential rather than simultaneous separations
  - Usually need < 2.0°C (must inject allelic ladder regularly)

- **Lower amount of DNA loaded** (injection = nL vs µL) and thus detection sensitivity must be better

- Electrokinetic injection enables **dye artifacts** (blobs) to enter the capillary or microchip CE channel and thus possibly interfere with STR allele interpretation

More Differences between CE and Gels...

- Filling the capillary (or microchip CE channel) is analogous to pouring a gel into a tiny tube...

- Must be more clean around a CE system
  - Because the capillaries (µCE channels) are small, particles of dust or urea crystals can easily plug them
  - Tips of capillary cannot dry out (once buffer solutions have been run through them) for the same reasons

- Bubbles are a BIG problem in CE as they can easily block current flow in the capillary...

---

**Capillary Electrophoresis (CE)**

- **Fill with Polymer Solution**
- **Argon Ion Laser**
- **50-100 µm x 27 cm**
- **DNA Separation occurs in minutes...**
- **5-20 kV**
- **Burn capillary window**
- **Data Acquisition and Analysis**
- **Sample tray**

---

**Typical Instruments Used for STR Typing**

- **GeneAmp 9700**
- **Thermal Cycler for PCR Amplification**

- **Singe capillary**
- **16-capillary array**

**ABI 310**
- **ABI 3100 → 3130xl**
  - **16 capillaries**
  - **1st purchased in April 2001 as ABI 3100**
  - Upgraded to 3130xl in Sept 2005
  - Located in a different room (A230, now B219)
  - **2nd purchased in June 2002 as NT (B231)**

**ABI 3500**
- **8 capillaries**
- **Purchased Nov 2010 (B233)**

---

**Genetic Analyzers from Applied Biosystems**

<table>
<thead>
<tr>
<th>ABI Genetic Analyzer</th>
<th>Years Released for Human ID</th>
<th>Number of Capillaries</th>
<th>Laser</th>
<th>Polymer delivery</th>
<th>Other Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>377</td>
<td>(gel system)</td>
<td>1992-2003</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>PEF and color filter wheel for detection</td>
</tr>
<tr>
<td>377</td>
<td>(gel system)</td>
<td>1995-2005</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>CDD camera</td>
</tr>
<tr>
<td>310</td>
<td>1995-2005</td>
<td>16</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>syringe</td>
</tr>
<tr>
<td>3100</td>
<td>2000-2005</td>
<td>96</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>Syringe</td>
</tr>
<tr>
<td>3100-Avant</td>
<td>2002-2007</td>
<td>4</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>Syringe</td>
</tr>
<tr>
<td>3130</td>
<td>2003-2011</td>
<td>4</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>Pump</td>
</tr>
<tr>
<td>3130xl</td>
<td>2003-2011</td>
<td>16</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>Pump</td>
</tr>
<tr>
<td>3500</td>
<td>2010-2010</td>
<td>8</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>Pump</td>
</tr>
<tr>
<td>3500xl</td>
<td>2010-2010</td>
<td>24</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>Pump</td>
</tr>
<tr>
<td>3700</td>
<td>2002-2003</td>
<td>96</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>Syringe</td>
</tr>
<tr>
<td>3730</td>
<td>2005-2006</td>
<td>48</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>Pump</td>
</tr>
<tr>
<td>3730xl</td>
<td>2005-2006</td>
<td>96</td>
<td>2</td>
<td>(He-Ne) Ar</td>
<td>Pump</td>
</tr>
</tbody>
</table>

Information courtesy of Michelle S. Shepherd, Applied Biosystems, Life Technologies.
**DNA Samples Run at NIST**

we have processed >100,000 samples (from 1996-present)

- **STR kits**
  - Identifier, PP16, PP16HS, Identifier Plus, Identifier Direct, Profiler Plus, Collifer, SGM Plus, ESi/ESX 17, SE33 monoplex
- **Research & development on new assays**
  - STRs: Y-STR 20plex, MeowPlex, miniSTRs, 26plex
  - SNPs: SNaPshot assays: mtDNA (one 10plex), Y-SNPs (four 6plexes), Orchid SNPs (twelve 6plexes), ancestry SNPs (two 12plexes), SNPtronID (one 29plex), SNPPlex (one 48plex)
- **DNA sequencing**
  - Variant allele sequencing

We have a unique breadth and depth of experience with these instruments...

---

**Analytical Requirements for STR Typing**


- Fluorescent dyes must be spectrally resolved in order to distinguish different dye labels on PCR products
- PCR products must be spatially resolved – desirable to have single base resolution out to >350 bp in order to distinguish variant alleles
- High run-to-run precision – an internal sizing standard is used to calibrate each run in order to compare data over time

---

**Review Article on STRs and CE**


---

**Steps in STR Typing with ABI 310/3100**


---

**Detection with Multiple Capillaries**


---

**Process Involved in 310/3100 Analysis**

- Separation
  - Capillary – 50um fused silica, 43 cm length (36 cm to detector)
  - POP-4 polymer – Polydimethyl acrylamide
  - Buffer - TAPS pH 8.0
  - Denaturants – urea, pyridilidione
- Injection
  - electrokinetic injection process (formamide, water)
  - importance of sample stacking
- Detection
  - fluorescent dyes with excitation and emission traits
  - CCD with defined virtual filters produced by assigning certain pixels
Separation

Ohm’s Law

• \( V = IR \) (where \( V \) is voltage, \( I \) is current, and \( R \) is resistance)
• Current, or the flow of ions, is what matters most in electrophoresis
• CE currents are much lower than gels because of a higher resistance in the narrow capillary
• CE can run a higher voltage because the capillary offers a higher surface area-to-volume ratio and can thus dissipate heat better from the ion flow (current)

DNA and Electrophoresis

“From a practical point of view it is disappointing that electrophoresis cannot be used to fractionate or analyze DNA’s on the basis of size” Olivera, Biopolymers 1964, 2, 245

\[ \mu_{ep} = q/6\pi\eta r \]
small ions with high charge move fastest

A T G C
PO⁻ PO⁻ PO⁻

As size increases so does charge!

Separation Issues

• Electrophoresis buffer –
  − Urea for denaturing and viscosity
  − Buffer for consistent pH
  − Pyrolidinone for denaturing DNA
  − EDTA for stability and chelating metals
• Polymer solution -- POP-4 (but others work also)
• Capillary wall coating -- dynamic coating with polymer
  − Wall charges are masked by methyl acrylamide
• Run temperature -- 60 °C helps reduce secondary structure on DNA and improves precision. (Temperature control affects DNA sizing)

What is in POP-4 and Genetic Analyzer Buffer?

| Improved single-strand DNA sizing accuracy in capillary electrophoresis |
| Barrett B. Rosenblatt, Frank Ots, Steve Meschen and Ben Johnson |
| US Patent 5,552,028 covers POP-4 synthesis |

POP-4 (4% poly-dimethylacrylamide, 8 M urea, 5% 2-pyrrolidinone)

Running buffer contains 100 mM TAPS and 1 mM EDTA (adjusted to pH 8.0 with NaOH) TAPS = N-Tris(hydroxymethyl)methyl-3-amino propane sulfonic acid

Capillary Wall Coatings Impact DNA Separations

**Electrophoretic flow**

DNA$^+$

Capillary Wall

SiOH

SiO$^-$$ + H^+$

**Electroosmotic flow (EOF)**

Solvated ions drag solution towards cathode in a flat flow profile

---

### How to Improve Resolution?

1. Lower Field Strength
2. Increase Capillary Length
3. Increase Polymer Concentration
4. Increase Polymer Length

*All of these come at a cost of longer separation run times*

---

### Impact of Capillary Length and Polymer Concentration on DNA Sequencing Resolution

<table>
<thead>
<tr>
<th>Capillary Length</th>
<th>Polymer Concentration</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>310 POP4-20min (36cm)</td>
<td>3%</td>
<td>Good</td>
</tr>
<tr>
<td>310 POP4-30min (36cm)</td>
<td>3%</td>
<td>Good</td>
</tr>
<tr>
<td>310 POP6-30min (36cm)</td>
<td>3%</td>
<td>Poor</td>
</tr>
<tr>
<td>310 POP6-50min (36cm)</td>
<td>3%</td>
<td>Poor</td>
</tr>
<tr>
<td>310 POP6-120min (50cm)</td>
<td>3%</td>
<td>Poor</td>
</tr>
<tr>
<td>310 POP7-120min (80cm)</td>
<td>3%</td>
<td>Poor</td>
</tr>
</tbody>
</table>

Data collected at NIST by Tomohiro Takamaya (Japanese guest researcher, fall 2007)

---

### Injection

- **Electrokinetic Injection Process**
  - Amount of DNA injected is inversely proportional to the ionic strength of the solution
  - Salty samples result in poor injections

---

### Capillary and Electrode Configurations

- **Single-Capillary**
  - PCR products in formamide or water

- **Multi-Capillary**
  - Sample Tube
  - Electrode
  - DNA
Capillaries

ABI 310
Electrode adjacent to capillary

ABI 3100
Individual electrode surrounds each capillary

Sample Conductivity Impacts Amount Injected

\[ [\text{DNA}_{\text{inj}}] = \frac{E(t(r^2)}{(\mu_{\text{ep}} + \mu_{\text{eof}})}\frac{[\text{DNA}]_{\text{sample}}}{\lambda_{\text{sample}}} \]

- \([\text{DNA}]_{\text{inj}}\) is the amount of sample injected
- \(E\) is the electric field applied
- \(t\) is the injection time
- \(r\) is the radius of the capillary
- \(\mu_{\text{ep}}\) is the mobility of the sample molecules
- \(\mu_{\text{eof}}\) is the electroosmotic mobility

Comments on Sample Preparation

- Use high quality formamide (<100 \(\mu\)S/cm)
- Denaturation with heating and snap cooling is not needed (although most labs still do it…)
- Post-PCR purification reduces salt levels and leads to more DNA injected onto the capillary

Steps Performed in Standard Module

See J.M. Butler (2005) Forensic DNA Typing, 2nd Edition; Chapter 14

- Capillary fill – polymer solution is forced into the capillary by applying a force to the syringe
- Pre-electrophoresis – the separation voltage is raised to 10,000 volts and run for 5 minutes
- Water wash of capillary – capillary is dipped several times in deionized water to remove buffer salts that would interfere with the injection process
- Sample injection – the autosampler moves to position A1 (or the next sample in the sample set) and is moved up onto the capillary to perform the injection; a voltage is applied to the sample and a few nanoliters of sample are pulled onto the end of the capillary
- Water wash of capillary – capillary is dipped several times in waste water to remove any contaminating solution adhering to the outside of the capillary
- Water dip – capillary is dipped in clean water (position 2) several times
- Electrophoresis – autosampler moves to inlet buffer vial (position 1) and separation voltage is applied across the capillary; the injected DNA molecules begin separating through the POP-4 polymer solution
- Detection – data collection begins; raw data is collected with no spectral deconvolution of the different dye colors; the matrix is applied during Genescan analysis

Why MiniElute increases peak heights

- QIAGEN MiniElute reduces salt levels in samples causing more DNA to be injected
- Requires setting a higher stochastic threshold to account for the increased sensitivity

Stochastic Effects and Thresholds

When PCR amplifying low levels of DNA, allele dropout may occur.

Stochastic threshold must be raised.

False homozygote

Detection

Optics for ABI 310

Fluorescence

Methods for Fluorescently Labeling DNA

The polymerase chain reaction (PCR) is used to amplify STR regions and label the amplicons with fluorescent dyes using locus-specific primers.
ABI Fluorescent Dyes Used in Four-Color Detection

- **FAM** (blue)
- **JOE** (green)
- TAMRA (yellow)
- **ROX** (red)

Fluorescent Emission Spectra for ABI Dyes

<table>
<thead>
<tr>
<th>WAVELENGTH (nm)</th>
<th>Laser excitation (488, 514.5 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>520</td>
<td>540</td>
</tr>
<tr>
<td>560</td>
<td>580</td>
</tr>
<tr>
<td>600</td>
<td>620</td>
</tr>
<tr>
<td>640</td>
<td>660</td>
</tr>
</tbody>
</table>

NED is a brighter dye than TAMRA

Importance of Spectral Calibration

Before Color Separation

After Color Separation

Importance of Spectral Calibration

Before Color Separation

After Color Separation

Matrix with 4 Dyes on ABI 310

\[
\begin{align*}
I_{540} &= bx + gy + yz + rw \\
I_{560} &= bx + g + yz + rw \\
I_{580} &= bx + g + y + rw \\
I_{610} &= bx + g + yz + yw \\
\end{align*}
\]

Where

- **b** is the %blue labeled DNA
- **g** is the %green labeled DNA, etc.
- x,y,z,w are the numbers in the matrix (sensitivity to each color)

If you solve xyzw for each dye individually
Then you can determine dye contribution for any mixture

5 x 5 matrix for 5-dye analysis on ABI 310

![5 x 5 matrix for 5-dye analysis on ABI 310](image)
Virtual Filters Used in ABI 310

Visible spectrum range seen in CCD camera

<table>
<thead>
<tr>
<th>Filter</th>
<th>Blue</th>
<th>Green</th>
<th>Yellow</th>
<th>Red</th>
<th>Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>FL</td>
<td>FAM</td>
<td>JOE</td>
<td>TMR</td>
<td>CX R</td>
</tr>
<tr>
<td>C</td>
<td>6FAM</td>
<td>TET</td>
<td>HEX</td>
<td>ROX</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>5FAM</td>
<td>JOE</td>
<td>NED</td>
<td>ROX</td>
<td></td>
</tr>
<tr>
<td>G5</td>
<td>6FAM</td>
<td>VIC</td>
<td>NED</td>
<td>PET</td>
<td>LIZ</td>
</tr>
</tbody>
</table>

Commonly used fluorescent dyes

Filter sets determine what regions of the CCD camera are activated and therefore what portion of the visible light spectrum is collected.

Deciphering Artifacts from the True Alleles

 Biological (PCR) artifacts
 Stutter products

STR alleles

Dye blob
Stutter
Spike

Pull-up (bleed-through)

Blue channel
Green channel
Yellow channel
Red channel

NIST ABI 3100 Analysis Using POP-6 Polymer

High Resolution STR Typing

SNuPhos SNP Typing
(Coding Region mtSNP 11plex minisequencing assay)

mtDNA Sequencing (HV1)

Protocols Used for STR Typing

- Most forensic DNA laboratories follow PCR amplification and CE instrument protocols provided by the manufacturer
- **Comments**
  - Lower volume reactions may work fine and reduce costs
  - No heat denaturation/snap cooling is required prior to loading samples into ABI 310 or ABI 3100
  - Capillaries do not have to be thrown away after 100 runs
  - POP-4 polymer lasts much longer than 5 days on an ABI 310
  - Validation does not have to be an overwhelming task

Maintenance of ABI 310/3100/3130

- Syringe – leaks cause capillary to not fill properly
- Capillary storage & wash – *it dries, it dies!*
- Pump block – cleaning helps insure good fill
- Change the running buffer regularly

YOU MUST BE CLEAN AROUND A CE!
ABI 3500 Genetic Analyzer

New Features of the ABI 3500 CE
- an improved polymer delivery pump design,
- ready-to-use consumables and containers,
- Radio Frequency Identification (RFID) consumable tracking,
- quality control software features for rapid identification and re-injection of failed samples,
- increased throughput,
- new laser technology,
- reduced power requirements,
- peak height normalization,
- intuitive user technology, and integrated primary analysis software,
- improved peak height uniformity across capillaries, runs and instruments,
- 6-dye channel capability

• 3500 (8 capillary)
• 3500xl (24 capillary)

Details of the new ABI 3500

No lower pump block (fewer air bubbles)

Improved sealing for better temperature control (improved precision?)

Better seal around the detector

Reagents prepackaged with RFID tags

Primary Differences Between 31xx and 3500

31xx Instruments
- Argon ion (Ar+) lasers with 488/514 nm wavelengths for fluorescence excitation
- 220V power requirement
- Optimal signal intensity 1500-3000 RFU
- Data signal depressed 4-fold during data collection
- Currently validated and operational in most forensic laboratories (.fsa files)

3500 Instruments
- Single-line 505 nm, solid-state long-life laser
- Smaller footprint
- 110V power requirement
- Optimal signal intensity can approach 20,000-30,000 RFU
- Normalization of instrument-to-instrument signal variability
  - Ability to increase or decrease overall signal
- Requires the use of GeneMapper IDX v1.2 (.hid files)

ABI 3500 ‘Dash Board’ Data Collection

Tracks the numbers of samples for ‘QC purposes’

ABI 3500 Generates Excellent Data

STR typing with a 1:7 mixture using 36 cm array and POP4

DNA sequencing of an SE33 allele using 50 cm array and POP7
Troubleshooting: Strategies and Solutions

Identifiers Result on ABI 3500xl

40,002 RFU
32,763 RFU

No pull-up observed

NIST Calculated Cost per Sample for ABI 3130xl vs. 3500 and 3500xl Reagents

Running two plates per day (10 plates per week)

Bruce McCord’s Profiles in DNA Article

Troubleshooting Capillary Electrophoresis Systems

Troubleshooting: Amplification and Electrophoresis of the AmpFISTR® Kits

One of the key responsibilities of our Forensic Identification Field Application Specialist is to troubleshoot results obtained using any of the AmpFISTR® kits in any Applied Biosystems validated instrument platform.

The key to producing good DNA separations is to understand the principles underlying the injection, separation and detection of each allele.

External Factors

- Room temperature
  - Variations in room temperature can cause mobility shifts with band shifts and loss of calibration
  - Temperature is also important due to effects of high humidity on electrical conductance

- Cleanliness
  - Urea left in sample block can crystallize and catalyze further crystal formation causing spikes, clogs and other problems.
  - Best bet is to keep polymer in system and not remove or change block until polymer is used up.
Effect of temperature on allele size

Slope is 0.14 bases/degree centigrade
Therefore a small change in temperature has a big effect
(A 1-2 degree shift in temperature of the heat plate can produce an OL allele)


Temperature Effects
Off-Ladder "OL Alleles"

"OL alleles" - look at the 250 peak

"OL allele re-injected"
And the 250 peak...

<table>
<thead>
<tr>
<th>60</th>
<th>200</th>
<th>201</th>
<th>202</th>
<th>203</th>
<th>204</th>
<th>205</th>
<th>206</th>
<th>207</th>
<th>208</th>
<th>209</th>
<th>210</th>
<th>211</th>
<th>212</th>
<th>213</th>
<th>214</th>
<th>215</th>
<th>216</th>
<th>217</th>
<th>218</th>
<th>219</th>
<th>220</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>250</td>
<td>350</td>
<td>500</td>
<td>700</td>
<td>900</td>
<td>1100</td>
<td>1300</td>
<td>1500</td>
<td>1700</td>
<td>1900</td>
<td>2100</td>
<td>2300</td>
<td>2500</td>
<td>2700</td>
<td>2900</td>
<td>3100</td>
<td>3300</td>
<td>3500</td>
<td>3700</td>
<td>3900</td>
<td></td>
</tr>
</tbody>
</table>

Temperature Probes

- Refrigerator and freezer monitoring
- Room temperature monitoring
- USB Temperature Datalogger PLUS Software $79.00 (#DT-23-33-46)
- Frig/Freeze Monitors $240 (#DT-23-33-80 - USB Temperature-Datalogger $91.00 (Cole Parmer, Vernon Hills IL))

Monitoring Instrument Room Temperature Fluctuations

- Temperature Monitoring of two separate instrument rooms.
- Box area is a 24 hour period where temperature control is not stable.

Poor Temperature Control Causes DNA Sizing Imprecision

Use of Second Allelic Ladder to Monitor Potential Match Criteria Problems

- Urea sublimates and breaks down to ionic components - these find a path to ground
- Similarly wet buffer under a vial creates paths to ground
- Capillary windows must be clear or matrix effects will occur
- Laser will often assist in this process
- Vial caps will transfer low levels of DNA to capillary

Cleanliness

- Urea sublimates and breaks down to ionic components - these find a path to ground
- Similarly wet buffer under a vial creates paths to ground
- Capillary windows must be clear or matrix effects will occur
- Laser will often assist in this process
- Vial caps will transfer low levels of DNA to capillary
Carbon Trails

High Humidity or wet buffer vials can create other paths to ground

Keep Your System Clean!

Instrumental Factors

- **Optical System**
  - Sensitivity changes with age, capillary diameter, capillary cleanliness, instrument calibration

- **Fluidic System**
  - Effects of bubbles, dust, urea crystals, leaks in syringe and capillary ferrule

- **Matrix Calculations**
  - Changes in buffer, optics, sample dye can alter the software calibrations

- **Capillary Problems**
  - Chemisorbed materials on capillary surface can produce osmotic flow, DNA band broadening and inconsistent resolution (meltdowns)

The Detection Window

Make sure that the capillary window is lined up (if it is not, then no peaks will be seen)

Window may need to be cleaned with ethanol or methanol

Review Start of Raw Data Collection

These spikes resulted from buffer dilution with poor water. The problem disappeared when the HPLC grade water was purchased to dilute buffer and samples

Beware of Urea Crystals

Urea crystals have formed due to a small leak where the capillary comes into the pump block

Urea sublimes and can evaporate to appear elsewhere

Use a small balloon to better grip the ferrule and keep it tight

Pump block should be well cleaned to avoid problems with urea crystal formation

Buffer Issues

- The buffer and polymer affect the background fluorescence affecting the matrix

- Urea crystals and dust may produce spikes

- High salt concentrations may produce reannealing of DNA

- High salt concentrations affect current

- Low polymer concentrations affect peak resolution
Meltdowns can be permanent or transitory as we have seen these may result from sample contamination effects.

Does the capillary need to be replaced?

No! The next injection looks fine…

Meltdowns may be the result of:

- Bad formamide
- Excess salt in sample/renaturation
- Water in the polymer buffer
- Syringe leak or bottom out
- Poisoned capillary
- Conductive polymer buffer due to urea degradation
- Crack/shift in capillary window
- Detergents and metal ions

Troubleshooting benchmarks:

- Monitor run current
- Observe syringe position and movement during a batch
- Examine ILS (ROX) peak height with no sample
- Observe “250 bp” peak in GS500 size standard
- Monitor resolution of TH01 9.3/10 in allelic ladder and size standard peak shapes
- Keep an eye on the baseline signal/noise
- Measure formamide conductivity
- Reagent blank – are any dye blobs present?
- See if positive control DNA is producing typical peak heights (along with the correct genotype)

Measurement of Current:

- \( V/I = R \) where \( R \) is a function of capillary diameter, [buffer], and buffer viscosity
- In a CE system the voltage is fixed, thus changes in resistance in the capillary will be reflected in the current observed
- Air bubbles, syringe leaks, alternate paths to ground, changes in temperature, changes in zeta potential, and contamination, will be reflected in the current
- A typical current for a CE system with POP4 buffer is 8-12 \( \mu A \) (microamps)

Syringe Travel:

- The ABI 310 instrument also keeps track of the position of the syringe (in the log file)
- Depending on the resistance to flow, the syringe will travel different lengths
- Syringe leaks may be reflected in a longer distance traveled prior to each injection
- These leaks occur around the barrel of the syringe and at the connection to the capillary block
Use of ABI 310 Log File to Monitor Current and Syringe Travel

Current Syringe Position

Manually filled syringes replaced by mechanical pump with polymer supplied directly from bottle

Measuring Formamide Conductivity

Dye Blobs in the Negative Control Sample

Conclusion:
Troubleshooting is more than following the protocols

It means keeping watch on all aspects of the operation
1. Monitoring conductivity of sample and formamide
2. Keeping track of current and syringe position in log.
3. Watching the laser current
4. Watching and listening for voltage spikes
5. Monitoring room temperature and humidity

The key is to measure the bottle when it comes in or buy the good stuff and immediately pipette it out into small tubes with or without ROX already added. Then freeze the tubes.

Do not ever open a cold bottle of formamide. Water will condense inside and aid in the formation of conductive formic acid.
Acknowledgments

Funding from an interagency agreement between the National Institute of Justice and the NIST Office of Law Enforcement Standards and also from the FBI

Many wonderful collaborators from industry, university, and government laboratories.

Bruce McCord (Florida International University) for many of the slides

Thank you for your attention

Contact Information

John Butler
NIST Fellow
Group Leader of Applied Genetics
john.butler@nist.gov
+1-301-975-4049

http://www.cstl.nist.gov/biotech/strbase

Our team publications and presentations are available at: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm